भारतीय मानक

Indian Standard

IS 3025 (Part 44) : 2023

जल एवं अपशिष्ट जल के नमूने लेना और परीक्षण (भौतिक एवं रसायनिक) की पद्धतियाँ

भाग 44 बायोकेमिकल ऑक्सीजन डिमांड (बीओडी)

(दूसरा पुनरीक्षण)

Methods of Sampling and Test (Physical and Chemical) for Water and Wastewater

Part 44 Biochemical Oxygen Demand (BOD)

(Second Revision)

ICS 13.060.50

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BUREAU OF INDIAN STANDARDS
मानक भवन, 9 बहादुर शाह ज़फर मार्ग, नई दिल्ली - 110002
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI - 110002
www.bis.gov.in www.standardsbis.in

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Water Quality Sectional Committee, CHD 36

FOREWORD

This Indian Standard (Part 44) (Second Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Water Quality Sectional Committee had been approved by the Chemical Division Council.

Biochemical Oxygen Demand (BOD) is the most important parameter to determine the degree of pollution in lakes and streams at any time and their self-purification capacities, assess the biodegradable organic load of the wastewaters for design of wastewater management system and thereafter to evaluate the efficiency of the same. As per definition BOD of a sample is defined as the amount of oxygen required by the micro-organisms to oxidise the organic matter by aerobic microbial decomposition to stable inorganic forms at some standard time and temperature.

Biochemical oxygen demand (BOD) test uses standard laboratory procedures to determine the relative oxygen requirements of waters, wastewaters, effluents, etc. There are a number of variations to the oxygen demand test prescribed in this standard. These include using shorter or longer incubation periods, higher temperatures, etc.

In a tropical country like India, the temperature of surface water in rivers, lakes, etc., vary from 20 °C to 35 °C in different seasons and in different parts of the country. The average aquatic temperature in our country is around 27 °C . Therefore the incubation temperature has been set at 27 °C and the incubation period as 3 days with an assumption that most of the carbonaceous organic demand is satisfied during this period.

The Technical Committee responsible for formulation of IS 2488 (Part 1): 1966 Methods of sampling and test for industrial effluents, Part 1 and IS 3025 : 1964 ‘Methods of sampling and test (physical and chemical) for water used in industry’ decided to revise the standard and publish it in separate parts. This standard superseded **12** of IS 2488 (Part 1) : 1966 Methods of sampling and test for industrial effluents, Part 1 and **53** of IS 3025 : 1964 Methods of sampling and test (physical and chemical) for water used in industry and was one among the different parts published under IS 3025 series of standards. The first revision of this standard was published in 1986.

In this second revision the following major modifications have been made:

a) Amendment No. 1 has been incorporated; and

b) Test procedure (Clause **9**) has been modified.

In the preparation of this standard, considerable assistance has been derived from Standard Methods for Examination of Water and Wastewater published by American Public Health Association, Washington, USA, 23rd edition.

The composition of the committee responsible for the formulation of this standard is listed in Annex A.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 2022 ‘Rules for rounding off numerical values (*second revision*)’.

Indian Standard

METHODS OF SAMPLING AND TEST (PHYSICAL AND CHEMICAL) FOR WATER AND WASTEWATER

PART 44 BIOCHEMICAL OXYGEN DEMAND (BOD)

(Second Revision)

1 SCOPE

This standard (Part 44) prescribes oxygen depletion method based on bio-assay procedure for measurement of biochemical oxygen demand.

2 REFERENCES

The following standards listed below contain provisions which through reference in this text constitute provisions of this standard. At the time of publications, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards listed below:

IS No.	*Title*
IS 3025 (Part 38) : 1989	Methods of sampling and test (physical and chemical) for water and wastewater: Part 38 Dissolved oxygen
IS 7022 (Part 1) : 1973	Glossary of terms relating to water, sewage and industrial effluents: Part 1
IS 7022 (Part 2) : 1979	Glossary of terms relating to water; sewage and industrial effluents: Part 2
IS 17614 (Part 1) : 2021	Water quality — Sampling: Part 1 Guidance on the design of sampling programmes and sampling techniques
IS 17614 (Part 3) : 2021	Water Quality — Sampling: Part 3 Preservation and handling of water samples

3 TERMINOLOGY

For the purpose of this standard, definitions given in IS 7022 (Part 1) and IS 7022 (Part 2) shall apply.

4 SAMPLING AND PRESERVATION

Sampling and sample preservation shall be done as prescribed in IS 17614 (Part 1) and IS 17614 (Part 2).

5 PRINCIPLE

5.1 The biochemical oxygen demand (BOD) test is based on mainly bio-assay procedure which measures the dissolved oxygen consumed by micro-organisms while assimilating and oxidizing the organic matter under aerobic conditions.

5.2 The standard test condition includes incubating the sample in an air tight bottle, in dark at as specified temperature for specific time.

6 APPARATUS

6.1 Incubation Bottles

6.1.1 300 ml capacity narrow neck special BOD bottles with planed mouth with ground glass stoppers. New bottles should be cleaned with 5 N hydrochloric acid or sulphuric acid followed by rinsing with distilled water. In normal use, bottles once used for Winklers procedure should only be rinsed with tap water followed by distilled water.

6.1.2 During incubation (if incubator is used) to ensure proper sealing, time to time, add water to the flared mouth of the bottle.

6.2 Water Bath or Air Incubator

Air incubation with thermostatically controlled 27 °C ± 1 °C. Avoid light to prevent possibility of photosynthetic production of oxygen.

NOTE – Thermostatically controlled at 27 °C ± 1 °C water bath with continuous stirring may be preferred.

7 REAGENTS

7.1 Phosphate Buffer Solution

Dissolve 8.5 g potassium di-hydrogen phosphate (KH_2PO_4), 21.75 g potassium hydrogen phosphate

(K_2HPO_4), 33.4 g disodium hydrogen phosphate ($Na_2HPO_4.7H_2O$) and 1.7 g ammonium chloride (NH_4Cl) in about 500 ml distilled water and dilute to 1 litre. *p*H of the solution should be around 7.2 without any further adjustment.

7.2 Magnesium Sulphate Solution

Dissolve 22.5 g magnesium sulphate ($MgSO_4.7H_2O$) in distilled water and dilute to 1 litre.

7.3 Calcium Chloride Solution

Dissolve 27.5 g calcium chloride in distilled water and dilute to 1 litre.

7.4 Ferric Chloride Solution

Dissolve 0.25 g hydrated ferric chloride ($FeCl_3.6H_2O$) in distilled water and dilute to 1 litre.

7.5 Acid and Alkali Solution

1 N sodium hydroxide and 1 N sulphuric acid for neutralization of samples.

NOTE — Any of the above solutions showing any sign of biological growth may be discarded.

7.6 Glucose-Glutamic Acid (GGA) Solution

Dry reagent grade glucose and reagent grade glutamic acid at 103 °C for 1 h. Add 150 mg of glucose and 150 mg of glutamic acid to distilled water and dilute to 1 litre. Prepare fresh immediately before use.

7.7 Other Reagents for Dissolved Oxygen Measurement

7.7.1 *Manganous Sulphate Solution*

Dissolve manganese sulphate (480 g of $MnSO_4.4H_2O$ or 400 g of $MnSO_4.2H_2O$ or 364 g of $MnSO_4.H_2O$) in freshly boiled and cooled water, filter and make up to 1 000 ml. The solution should not give blue colour by addition of acidified potassium iodide solution and starch.

7.7.2 *Alkali-Iodide Azide-Reagent*

Dissolve 500 g of sodium hydroxide (or 700 g of potassium hydroxide) and 135 g of sodium iodide (or 150 g of potassium iodide) in freshly boiled and cooled water and dilute to1 litre.

Dissolve 10 gm of sodium azide in 40 ml of distilled water and add this with constant stirring to the cool alkaline iodide solution.

7.7.3 *Sulphuric Acid, Concentrated*

7.7.4 *Starch Indicator*

Dissolve 2 g of starch and 0.2 g of salicylic acid as preservative, in 100 ml of hot distilled water.

7.7.5 *Sodium Thiosulphate Stock Solution*

Dissolve approximately 25 g of sodium thiosulphate ($Na_2S_2O_3.5H_2O$) in boiled distilled water and make up to 1 000 ml. Add 1 g of sodium hydroxide to preserve it.

7.7.6 *Standard Sodium Thiosulphate Solution*

Dissolve 250 ml of stock solution (**7.7.5**) in boiled distilled water and make up to 1 litre and standardize sodium thiosulphate against known standard before use.

7.7.7 *Potassium Dichromate Solution,* $K_2Cr_2O_7$, 0.025 N

Dissolve 1.225 9 gm of $K_2Cr_2O_7$ (previously dried at 150 °C for 2 h) in distilled water and dilute to 1 litre.

7.7.8 *Potassium Iodate Solution,* 0.025 N

Dissolve 0.892 5 gm. of KIO_3 (dried at 120 °C to 140 °C) in distilled water and dilute to 1 litre.

8 INTERFERENCES

Oxidizing agents give positive interferences and reducing agents give negative interferences. Nitrite interference is removed by adding sodium azide to alkaline iodide solution.

9 PROCEDURE

9.1 Preparation of Dilution Water

Aerate the required volume of distilled water in a container by bubbling compressed air for 8 h to 12 h to attain dissolved oxygen saturation. Let it stabilize for 4 h at room temperature (around 27 °C). Add 1 ml each of phosphate buffer solution, magnesium sulphate solution, calcium chloride solution and ferric chloride solution before aerating or may be added alternately at the time of preparing the sample dilutions.

9.2 Dilution Water Check

With each batch of dilution water, incubate at least two or more bottles of dilution water containing nutrient, mineral and buffer solutions without seed to verify the quality of water. One set of Dilution

water checks must be analyzed with each batch of samples. The dilution water blank serves as a check on the quality of unseeded dilution water and cleanliness of incubation bottles. Determine initial and final Dissolved Oxygen (DO) for each bottle and average the results. The average DO uptake in 3 days must not be greater than 0.2 mg/l and preferably ≤ 0.1 mg/l before making seed corrections. If average dilution water blank is greater than 0.2 mg/l, record the data and clearly identify such samples in data records

9.3 Addition of Seed to Dilution Water

Use treated sewage or commercially available microbial seed mixture for seeding purposes.

When treated sewage is not available, use supernatant from domestic waste water after settling at room temperature for at least 1 h but not longer than 36 h. The preferred seed is effluent or mixed liquor from a biological treatment system processing the waste.

NOTE — Seeding's not required for domestic sewage or surface water samples.

The DO uptake attributable to the seed added to each BOD bottle generally should be between 0.6 mg/l and 1.0 mg/l but the amount of seed added should be adjusted from this range to that required to provide glucose-glutamic acid (GGA) check results of 198 mg/l ± 30.5 mg/l. For example, if 1 ml of seed suspension is required to achieve 198 mg/l ± 30.5 mg/l BOD in the glucose-glutamic acid check, then add 1 ml of seed suspension in each sample bottle.

9.4 Neutralization

Neutralize the sample to *p*H around 7.0 using alkali or acid of such strength that the quantity of reagent does not dilute the sample by more than 0.5 percent.

9.5 Sample Volume and Dilution Techniques

Four types of dilutions were suggested for use:

9.5.1 *Method 1 Dilutions Based on Expected BOD and COD Values*

On the basis of Chemical Oxygen Demand (COD), determine expected BOD. Use the following formula for calculating sample volume:

Sample volume in ml, per l dilution =

$$\frac{X}{\text{expected BOD}} \times 1\,000$$

For keeping 2 dilutions take X = 2.5 and 4.0

For single dilution take X = 3.0 or 3.5.

9.5.2 *Method 2 Direct Dilution if the Sources are Known*

If unknown concentrations is given the following idea can be used:

a) Strong effluents 0.1 percent to 1 percent;
b) Raw or settled waste 1 percent to 5 percent;
c) Biologically treated water 5 percent to 25 percent; and
d) River water/surface waters 25 percent to 100 percent.

9.5.3 *Method 3 Percent Dilution of Sample in* 1 litre *Measuring Cylinders and Siphoning Techniques*

Fill a 1 litre volumetric flask with approximately 300 ml dilution water as prepared. Add the specific volume of sample in it as per dilution technique given in Table 1. If the dilution water is not feeded with nutrients during aeration, then add 1 ml each of phosphate buffer solution, magnesium sulphate solution, calcium chloride solution and ferric chloride solution one by one in the volumetric flask. If the sample requires seeding, add the requisite volume of seed which meets the GGA check of 198 °C ± 30.5 °C as mentioned in Table 2 and dilute to the mark with the dilution water by siphoning from the dilution water container. Mix well. Rinse three BOD bottles with the diluted sample and fill up these bottles with the diluted sample by siphoning from the volumetric flask. Stopper the bottles immediately after removing the air bubbles. Repeat the same process for other dilutions of the sample.

Table 1 Statistical Table for Taking Suitable Dilution in to 1 litre Flask
(*Clause* 9.5.3)

Sl No.	Estimated BOD Range, mg/l (based on COD)	Percent of Sample to Dilute to 1 000 ml
(1)	(2)	(3)
i)	20 000 – 70 000	0.01
ii)	10 000 - 35 000	0.02
iii)	4 000 - 14 000	0.05
iv)	2 000 - 7 000	0.1
v)	1 000 - 3 500	0.2
vi)	400 - 1 400	0.5
vii)	200 - 700	1.0
viii)	100 - 350	2.0
ix)	40 - 140	5.0
x)	20 - 70	10
xi)	10 - 35	20
xii)	4 - 14	50
xiii)	0 - 7	100

NOTE — Suitable dilutions for at least two sets are to be taken i.e if BOD is in the range between 10 mg/l to 70 mg/l then samples with 10 percent and 20 percent must be taken for analysis.

Table 2 Method for Direct Pipetting the Samples into BOD Bottles if Large Number of Samples are Handled
(*Clause* 9.5.3)

Sl No.	Range of BOD Expected mg/l	Volume of Sample to 300 ml BOD Bottle, in ml
(1)	(2)	(3)
i)	30 000 - 105 000	0.02
ii)	12 000 - 42 000	0.05
iii)	6 000 - 21 000	0.1
iv)	3 000 - 10 500	0.2
v)	1 200 - 4 200	0.5
vi)	600 - 2 100	1.0
vii)	300 - 1 050	2.0
viii)	120 - 420	5.0
ix)	60 - 210	10
x)	30 - 105	20
xi)	12 - 42	50
xii)	6 - 21	100
xiii)	0 - 7	300

NOTE — The dilutions taken for BOD should result in a residual DO of at least 1 mg/l and a DO uptake of at least 2 mg/l after 3 days' incubation.

9.5.4 *Method for Direct Pipetting the Samples into BOD Bottles if Large Number of Samples are Handled*

Samples of natural surface water bodies like River, Lake and marine, generally do not require seeding and dilution due to naturally available microbiological population and low BOD values. For such samples which are likely to have BOD less than 5 mg/l, BOD determination may be carried out as such (100 percent) without any dilution.

9.6 Use of Seed Control

For preparing a seed control, fill up a 1 litre volumetric flask partly with the dilution water, add 20 ml of seed (2 percent of freshly collected or stabilised settled sewage) in it and 1 ml each of the

nutrients and fill up to the mark with the dilution water. The volume of seed added may vary depending on the quality of seed used.

9.7 Sealing Bottles

Completely fill each bottle by adding enough dilution water so that insertion of stopper leaves no bubbles in the bottle. Mix sample by turning bottle manually several times. As a precaution against drawing air into the BOD bottles during incubation, use a water seal. Obtain satisfactory water seals by inverting bottles in a water bath or by adding water to the flared mouth of the BOD bottles. During incubation (if incubator is used), in order to ensure proper sealing, add water to the flared mouth of the bottle from time to time.

9.8 Sample Incubation

Incubate at 27 °C ± 1 °C the stoppered and sealed BOD bottles containing desired dilutions, seed controls, dilution water blanks and GGA checks. Exclude light to avoid algae growth in bottles during incubation.

9.9 Standardisation of Sodium Thiosulphate Solution using Potassium Iodate/Potassium Dichromate Solution

Take 25 ml of potassium iodate solution (0.025 N) or 25 ml of potassium dichromate solution (0.025 N) in a 250 ml. glass-stoppered conical flask and add 5 ml concentrated H_2SO_4 to it. Add 1 gm KI to it and keep it in the dark for a few min. Then titrate this against $Na_2S_2O_3$ solution adding starch towards the end of titration, when a pale straw colour appears. Continue titration till the solution becomes colourless. Determine the strength of sodium thiosulphate solution using the normality equation.

9.10 Determination of Initial Dissolved Oxygen (DO)

Determine initial DO for one bottle and keep two bottles with samples for incubation at 27 °C ± 1 °C for 3 days.

NOTE — DO shall be determined as per IS 3025 (Part 38).

9.11 Determination of Final DO

After 3 days incubation at 27 °C ± 1 °C, determine final DO in incubated bottles.

NOTE — DO shall be determined as per IS 3025 (Part 38).

10 CALCULATION

For each test bottle having 2.0 mg/l Min DO depletion and at least 1.0 mg/l residual DO, calculate BOD as follows:

10.1 When Sample is Undiluted and Direct Sample is Used for Fresh Surface Waters/Rivers and Less Than 5 mg/l

BOD, mg/l = DO, in mg/l before incubation – DO, in mg/l after incubation after 3 days

10.2 When Dilution Water is not Seeded but Suitable Volume of Sample is Taken in Less Polluted Waters

$$\text{BOD, mg/l} = \frac{D_1 - D_2}{V} \times 1\,000$$

10.3 When Dilution Water is Seeded

$$\text{BOD, mg/l} = \frac{(D_1 - D_2) - \{(B_1 - B_2)f\}}{V} \times 1\,000$$

where

D_1 = initial DO of sample in mg/l;

D_2 = DO of sample after incubation in mg/l;

B_1 = DO of seed control before incubation in mg/l;

B_2 = DO of seed control after incubation in mg/l;

f = ratio of seed in diluted sample to seed in control; [volume (ml) of seed in diluted sample/volume of seed in seed control]; and

V = sample volume (in ml).

NOTE — f may be used only when seed correction is to be applied.

11 GLUCOSE GLUTAMIC ACID CHECK

BOD being a bioassay test, is greatly influenced by factors like toxicants, poor seeding, etc. For periodical checking of these factors, use a mixture of 150 mg/l glucose and 150 mg/l glutamic acid as a standard check solution. Take five sets of 2 percent Glucose-glutamic acid solutions for the check. Add 1 ml, 2 ml, 3 ml, 4 ml and 5 ml seed in the 1 litre volumetric flasks containing the 2 percent GGA solutions respectively along with the nutrients and make up the volume with the dilution water. Determine the 3 days 27 °C BOD of 2 percent dilution of the glucose-glutamic acid standard check solutions as in **9**. If the average BOD value of the check solutions is outside the range of 198 mg/l ± 30.5 mg/l, reject any BOD determinations made with the seed and dilution water and seek the cause of the problem.

12 EXPRESSION OF RESULTS

BOD is expressed as mg/l, 3 days at 27 °C as given in **10** and round off the values as follows:

a) 0 to 10 up to first decimal; and
b) Above 10 – whole number.

ANNEX A

(*Foreword*)

COMMITTEE COMPOSITION

Water Quality Sectional Committee, CHD 36

Organization	*Representative(s)*
Chief Scientist, EPTRI, Hyderabad	SHRI N. RAVEENDHAR **(*Chairperson*)**
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In Personal Capacity *(1221, Mahatma Gandhi Road, P. O. - Haridevpur, Kolkata - 700082)*	SHRI SANJIB KUMAR GOSWAMI
In Personal Capacity *(S-168 A- Uppal Sothend, Sector 49, Sohna Road, Gurugram - 122018)*	SHRI RAKESH MALHOTRA
In Personal Capacity (*H. No. 1-78/2/S/121/1, Sathi Reddy nagar colony, Boduppal, Hyderabad, - 500092*)	SHRI N. MURALI MOHAN
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BUREAU OF INDIAN STANDARDS

Headquarters:

Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi 110002
Telephones: 2323 0131, 2323 3375, 2323 9402 *Website*: www.bis.gov.in

Regional Offices:		*Telephones*
Central	: 601/A, Konnectus Tower -1, 6th Floor, DMRC Building, Bhavbhuti Marg, New Delhi 110002	2323 7617
Eastern	: 8th Floor, Plot No 7/7 & 7/8, CP Block, Sector V, Salt Lake, Kolkata, West Bengal 700091	2367 0012 2320 9474
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